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Effects of glucocorticoid and mineralocorticoid on potassium transport in the rat medullary thick ascending limb of Henle's loop

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Effects of glucocorticoid and mineralocorticoid on potassium transport in the rat medullary thick ascending limb of Henle's loop. Potassium transport in the thick ascending limb is conducted by heterogeneous cells to opposite directions. Effects of glucocorticoids and mineralocorticoids on potassium transport of the rat medullary thick ascending limb (MTAL) were examined by in vitro microperfusion technique by measuring net potassium flux (J_K), and apparent potassium conductances in the apical and basolateral membranes of two cell populations. Segments of MTAL were obtained from four groups of rats: sham operated control rats, adrenalectomized rats (ADX), adrenalectomized rats treated with dexamethasone (DEX), and adrenalectomized rats treated with aldosterone (ALDO). Fractional urinary potassium excretion was reduced by ADX and partially recovered by either DEX or ALDO. J_K of the isolated perfused MTAL was markedly decreased by ADX from 5.13 to 1.94 pmol/min/mm. It was partially recovered by DEX (3.36 pmol/min/mm), but not by ALDO (2.07 pmol/min/mm). Random impalement of MTAL cells with a microelectrode in the control group revealed two cell populations; 76% was high basolateral conductance cell (HBC) and 24% was low basolateral conductance cell (LBC). In the ADX group, the basolateral potassium conductance of the HBC cell was markedly reduced, whereas the apical membrane potassium conductance of the HBC cell was increased. These changes were recovered in the DEX group, but not in the ALDO group. Potassium conductances of the apical and basolateral membranes in the LBC were unchanged. These findings suggest that potassium transport in the MTAL is regulated mainly by glucocorticoids which may predominantly act on the HBC. However, it remains to be established whether ADX causes a conversion of HBC to LBC or alters the basolateral K^+ conductance of the HBC.

Although it has been well established that the collecting duct plays an integral part in the regulation of potassium balance operated by the kidney [1], the potassium recycling within the loop of Henle may play an additional role in the urinary excretion of potassium [2, 3]. While potassium entry occurs in the descending limb by a passive manner, net potassium reabsorption occurs in the thick ascending limb of Henle's loop. The overall potassium transport in the latter segment may be determined by the balance between absorptive flux and secretory flux operated by two distinct types of cells [4–6].

Guggino [4] reported that in amphibian diluting segment there are functionally distinct two cell types as defined by the differences in potassium conductances in the basolateral and apical membranes: one had high basolateral and low apical membrane potassium conductances, while the other had low basolateral and high apical membrane potassium conductances. He designated the former as high basolateral membrane conductance cell (HBC) and the latter as low basolateral membrane conductance cell (LBC). Yoshitomi et al [5] subsequently reported that the similar functional cell heterogeneity also exists in the hamster medullary thick ascending limb (MTAL), and speculated that the HBC may contribute to the reabsorptive potassium flux whereas the LBC participates in the potassium secretion. More recently, Tsuruoka et al [6] provided circumstantial evidence in support of this notion: they reported that potassium is secreted in the cortical thick ascending limb where the LBCs predominate, whereas potassium is absorbed in the MTAL where the HBCs are the majority cell population.

The effects of adrenal steroids including gluco- and mineralocorticoids on the thick ascending limb have been explored by *in vitro* micropuncture [7–10] as well as *in vitro* microperfusion [11] techniques. In view of the cellular heterogeneity of the thick ascending limb of Henle's loop as mentioned above, the questions may be raised as to which cells are responsible for the target of the adrenal corticosteroids and which corticosteroids play major roles. To answer these issues, we perfused segments of the MTAL isolated from adrenalectomized rats treated with or without gluco- or mineralocorticoids. In the present paper, we present circumstantial evidence in support of the view that glucocorticoids act on the majority cell[1] of the MTAL to increase the basolateral membrane K^+ conductance and to decrease the apical K^+ conductance, contributing to an increase in net potassium reabsorption in the MTAL.

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[1] Because the membrane potassium conductances of the HBC cell of the rat MTAL are changed markedly by adrenalectomy, the nomenclature of HBC or LBC might not be appropriate. However, because we do not know at the present time whether adrenalectomy causes a conversion of HBC to LBC or simply changes the membrane K^+ conductances of HBC, the conventional terminology was used throughout this paper.

Methods

Animals preparation

Either male or female Sprague-Dawley rats (4 to 6 weeks old, weight 100 to 130 g) fed a standard laboratory chow were used. The animals were divided into four groups.

Group 1. Control group. Animals were sham-operated and received vehicle (sesame oil, s.c.) every day after operation.

Group 2. ADX group. Animals were adrenalectomized and then received only vehicle for 7 to 9 days.

Group 3. DEX group. Animals were adrenalectomized and then received dexamethasone for 7 to 9 days (1.2 μg/100 g body wt/day s.c.).

Group 4. ALDO group. Animals were adrenalectomized and then received aldosterone for 7 to 9 days (0.5 μg/100 g body wt/day s.c.).

Bilateral adrenalectomy or sham operation was performed under ether anesthesia seven to nine days before experiments. Then, animals of ADX, DEX, and ALDO groups were allowed to drink 0.9% NaCl *ad libitum*. One day before the experiment, each animal was transferred to a metabolic cage to measure urine volume and food or fluid intake for 24 hours. Dexamethasone and aldosterone were purchased from Sigma (St. Louis, MO, USA). They were dissolved in sesame oil at the concentrations of 12 μg/ml and 5 μg/ml, respectively.

In vitro microperfusion study

Rats were anesthetized with pentobarbital (40 mg/kg, i.p.). After taking blood samples from the abdominal aorta, both kidneys were removed and coronal slices were made. They were transferred to a dish containing modified Collins solution of the following composition (in mM); 14 KH_2PO_4, 14 K_2HPO_4, 15 KCl, 9 $NaHCO_3$, and 160 sucrose (pH 7.4), maintained at 4 to 5°C. Segments of the MTAL were isolated with fine forceps under a stereomicroscope.

The *in vitro* microperfusion method developed by Burg et al [12] was used as modified in our laboratory [5, 6, 13]. Isolated MTAL tubules were transferred to a perfusion bath mounted on an inverted microscope (IMT-2, Olympus, Tokyo) and perfused *in vitro* at 37°C. A system of the flowing-through bath was utilized to permit rapid exchange of the bathing fluid. The flow rate of the bathing fluid was ranged from 5 to 10 ml/min.

The composition of the basal solution used in this study was as follows (in mM); 110 NaCl, 5 KCl, 25 $NaHCO_3$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 10 Na acetate, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 8.3 glucose and 5 alanine. When potassium concentration in the bathing fluid or perfusate was increased from 5 to 50 mM, 45 mM NaCl was replaced with 45 mM KCl. The pH of these solutions was maintained at 7.4 by bubbling with 95% O_2-5%CO_2 gas.

Measurement of net potassium flux

Net potassium flux (J_K) of the MTAL was measured as follows. About 10 μCi/ml ^{14}C-methoxy inulin (NEN) was added to the perfusate as a volume marker. After transmural voltage (V_T) of the perfused tubule was stabilized, each three samples of tubular effluent were collected into constant volume pipette for measurements of net water flux (J_V) or J_K. Samples for J_V measurement were transferred to a vial containing 0.5 ml water, to which was added 3 ml scintillater solution with the composition of 4 mg Omnifluor/ml toluene:Triton-X100(2:1) solution to permit measurement of β-emission of ^{14}C on a β-counter (LSC-3500; Aloka, Tokyo, Japan). The constant volume pipette was calibrated at the end of each experiment by sampling the perfusate placed in the collecting pipette and counting according to the same procedure as was used for the measurement of tubular effluent. Net water flux was calculated as;

$$J_V = V_o(C^*_o/C^*_i - 1)/L,$$

where V_o is collection rate (nl/min), L is tubular length (mm), and C^*_o and C^*_i are concentrations of ^{14}C-methoxy inulin of collected fluid and the perfusate, respectively.

Samples for potassium measurement were transferred to plastic wells (Terasaki tissue culture plate, 3034 Micro Test; Falcon, Oxnard, CA, USA) filled with water equilibrated mineral oil. The perfusate and the standard solutions were treated similarly. The potassium concentration of these samples was measured by a ultramicro flame photometer (AFA-707-D; Apel, Tokyo, Japan) without dilution. For one sample of about 80 nl, the measurements were repeated at least three times by interposing the measurements of higher or lower potassium standard samples. The net potassium flux was calculated as:

$$J_K = (V_i[K^+]_i - V_o[K^+]_o)/L = [K^+]_iJ_V + ([K^+]_i - [K^+]_o)V_o/L,$$

where V_i and V_o are perfusion rate and collection rate, and $[K^+]_i$ and $[K^+]_o$ are K^+ concentration of the perfusate and the collected fluid, respectively. Positive value for J_K means net absorption.

Electrophysiological study

Transmural voltage (V_T) was measured by connecting a 3 M KCl agar bridge to saturated KCl reservoir where a calomel half cell electrode was placed. The electrode was connected to a dual channel electrometer (Duo 773; WP Instruments, CT, USA) and recorded on a two-pen chart recorder (R-301; Rikadenki, Tokyo, Japan). The circuit was connected to the bath with a 3 M KCl agar bridge, serving as a common ground.

Basolateral membrane voltage (V_B) was measured by random intracellular impalement of the epithelia of the perfused segment with a conventional microelectrode fabricated by a vertical puller (PE-2; Narishige, Tokyo, Japan). Electrodes were filled with 0.5 M KCl (100 to 200 MΩ) and connected to another channel of the electrometer via a holder that contained Ag-AgCl pellet. The position of electrode was controlled with manipulators (MO-102M; Narishige) fixed on the stage of the inverted microscope. Impalement of an electrode was conducted by table tapping or electrical current oscillation. Apical membrane voltage (V_A) was calculated as $V_A = V_B - V_T$. The criteria for acceptable impalement were as follows: (1) V_B changed abruptly upon impalement; (2) V_B reached a maximum level within a few minutes, and was stable for at least five minutes; and (3) V_B recovered to the level of ±3 mV after withdrawal of the electrode.

The membrane voltage deflections (ΔV_A or ΔV_B) induced by abrupt changes in K^+ concentration from 5 to 50 mM in either luminal or bathing fluid were assumed to represent apparent K^+ conductances.

Measurement of blood or urine samples

Blood or urine concentration of Na^+, K^+ and Cl^- was measured with an autoanalyzer (Electroder; A&T, Chiba, Japan). Creatinine concentration was measured by another autoanalyzer

Table 1. Summary of metabolic balance studies in four groups of rats

	Control (5)	ADX (6)	DEX (5)	ALDO (5)
ΔBW *g/wt*	27.1 ± 1.4	23.0 ± 1.1[a]	20.6 ± 0.9[a]	21.0 ± 0.9[a]
Urine volume *ml/day*	7.0 ± 0.4	20.8 ± 2.2[a]	21.6 ± 2.4[a]	20.8 ± 1.6[a]
Food intake *g/day*	17.7 ± 0.7	26.4 ± 0.7[a]	27.1 ± 0.5[a]	23.7 ± 1.6[ab]
Fluid intake *ml/day*	10.6 ± 0.2	12.5 ± 2.3	11.0 ± 1.3	11.9 ± 0.5
S_{Na} *mEq/liter*	140.7 ± 0.3	141.6 ± 0.9	143.0 ± 2.1	139.3 ± 0.9
S_K *mEq/liter*	3.9 ± 0.1	5.3 ± 0.3[a]	4.5 ± 0.1[ab]	4.7 ± 0.1[ab]
S_{Cr} *mg/dl*	0.40 ± 0.04	0.37 ± 0.03	0.36 ± 0.02	0.37 ± 0.02
FE_{Na} *%*	0.23 ± 0.05	1.39 ± 0.15[a]	1.05 ± 0.11[ab]	0.84 ± 0.11[ab]
FE_K *%*	23.6 ± 4.3	14.4 ± 1.6[a]	18.2 ± 1.9[ab]	22.1 ± 1.3[b]
U_{Na}/U_K	0.43 ± 0.03	2.63 ± 0.17[a]	1.44 ± 0.18[ab]	1.12 ± 0.08[ab]
S-aldosterone *ng/dl*	4.3 ± 0.3	<1.6[a]	<1.6[a]	4.5 ± 0.3
S-corticosterone *ng/dl*	953.3 ± 73.5	<50[a]	<50[a]	<50[a]

Abbreviations are: ADX, adrenalectomized; DEX, dexamethasone; ALDO, aldosterone; ΔBW, increase in body weight; S, serum concentration; FE, fractional excretion. Numbers in parentheses represent numbers of experiments.

[a] $P < 0.05$ compared to values in the control

[b] $P < 0.05$ compared to values in ADX

Table 2. Net potassium fluxes (J_K) and transmural voltage (V_T) in the MTAL from four groups of rats

	Control (7)	ADX (8)	DEX (8)	ALDO (8)
Length *mm*	0.61 ± 0.03	0.54 ± 0.04	0.53 ± 0.02	0.53 ± 0.02
Vi *nl/min*	4.29 ± 0.28	4.60 ± 0.19	4.95 ± 0.13	4.87 ± 0.10
J_V *nl/min/mm*	0.00 ± 0.03	−0.01 ± 0.03	−0.04 ± 0.02	0.01 ± 0.01
Ki *mEq/liter*	4.79 ± 0.03	4.91 ± 0.05	4.91 ± 0.01	5.01 ± 0.03
Ko *mEq/liter*	4.03 ± 0.11	4.66 ± 0.05	4.54 ± 0.03	4.79 ± 0.04
J_K *pmol/min/mm*	5.13 ± 0.30	1.94 ± 0.25[a]	3.36 ± 0.20[ab]	2.07 ± 0.14[a]
V_T *mV*	2.8 ± 0.1	1.3 ± 0.1[a]	3.3 ± 0.1[ab]	1.6 ± 0.2[a]

Abbreviations are: L, tubular length; Vi, perfusion rate; J_V, net water flux; Ki and Ko; potassium concentration in perfusate and collected fluid; J_K, net potassium flux; V_T, transmural voltage. Numbers in parentheses represent numbers of experiments.

[a] $P < 0.05$ compared to values in the control

[b] $P < 0.05$ compared to values in ADX

(Autoanalyzer 736, Hitachi, Japan). Aldosterone and corticosterone concentrations were measured by radioimmunoassay (Diagnostic Products Corporation, CA, USA, and ICN Biomedicals, Inc. CA, USA, respectively).

Statistical analysis

All data are expressed as mean ± SE. Statistical analyses were performed by ANOVA. $P < 0.05$ was regarded as significant.

Results

Clearance study

The results of metabolic balance studies conducted in four groups of rats are summarized in Table 1. In the groups of ADX, DEX, and ALDO, urine volume was markedly increased as compared to that of the control group. The gain of body weight was smaller in these groups than that in the control, although the food intake was larger. The fluid intake was unchanged. Serum sodium and creatinine concentrations were unchanged. Serum potassium concentration was increased by adrenalectomy. This increase was partially improved by the treatment with either dexamethasone or aldosterone.

The fractional urinary excretion of sodium (FE_{Na}) and the urinary Na/K ratio ($U_{Na/K}$) was increased by adrenalectomy and partially improved by the treatment with dexamethasone or aldosterone. The fractional urinary excretion of potassium (FE_K) was markedly increased by adrenalectomy, partially improved by administration of dexamethasone, and completely normalized by administration of aldosterone.

To confirm whether adrenalectomy was perfect, serum concentrations of aldosterone and corticosterone were measured. As also shown in Table 1, serum concentrations of both steroids were undetectable in adrenalectomized rats. In the adrenalectomized rats supplemented with aldosterone, serum aldosterone level became normal.

Net potassium flux

Net potassium fluxes (J_K) and transmural voltage (V_T) were determined in perfused segments of the MTAL isolated from the kidneys of four groups of rats. The results are summarized in Table 2. Individual data are shown in Figure 1. In the control group, J_K was 5.13 pmol/min/mm and V_T was 2.8 mV. These values are comparable to those reported for the hamster MTAL [5, 6]. Adrenalectomy caused marked reductions of both J_K and V_T. By treatment with dexamethasone, the decreased J_K was improved and V_T became slightly higher than the control value. However the recovery of J_K was not complete because J_K was significantly lower than that in the control. By contrast, both J_K and V_T were unchanged by the treatment with aldosterone.

Apparent potassium conductance

Random intracellular impalement with a microelectrode was conducted in the MTAL obtained from four groups of rats. In the control group, we impaled 25 cells from 20 MTALs. As shown in representative tracings of V_T and V_B (Fig. 2A), random cell impalement revealed that there are two cell populations with respect to the voltage deflections of the apical and basolateral membrane voltages upon abrupt changes in potassium concentration in the luminal or the bathing fluid. Membrane conductances of eighteen HBCs were comparable to those reported for the hamster MTAL [5, 6], having a high basolateral and a low apical membrane potassium conductance (Fig. 3A). In these cells, the deflection of the basolateral membrane voltage upon abrupt increase in potassium concentration of the bathing fluid (ΔV_B)

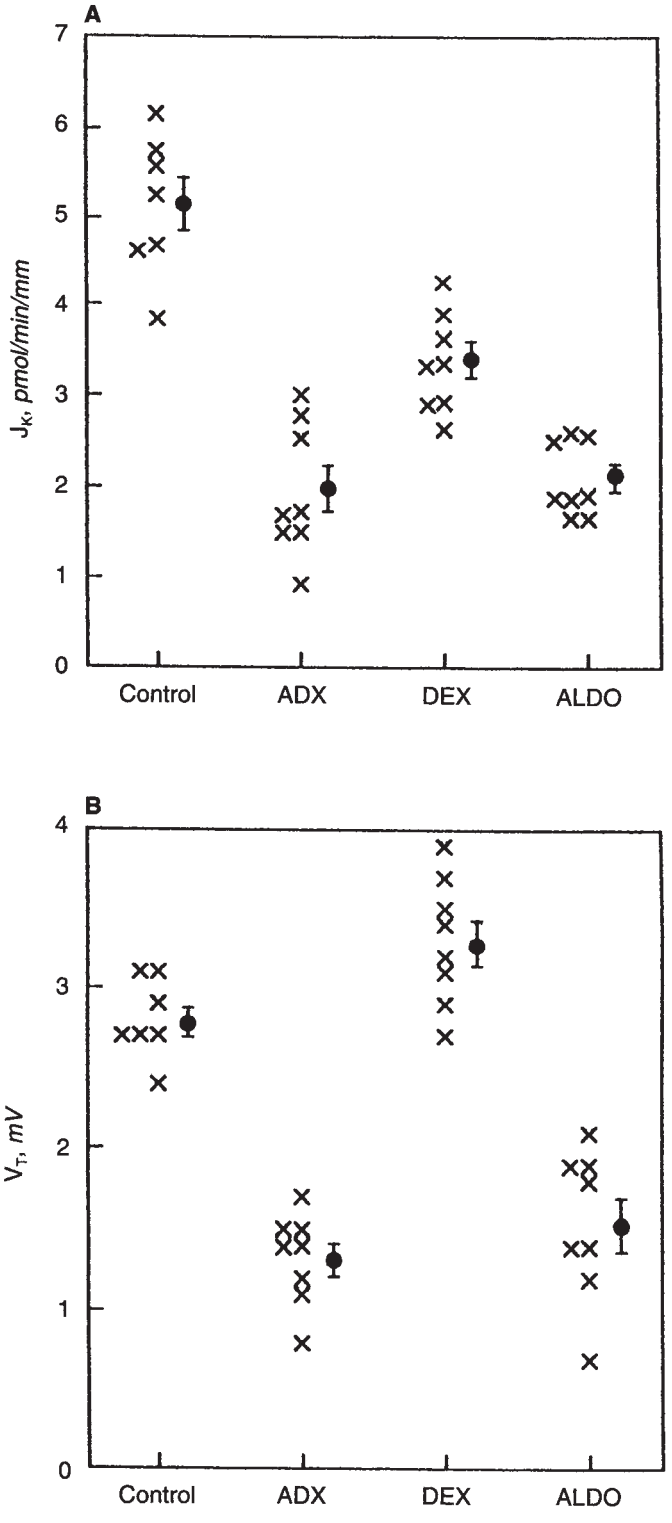

Fig. 1. *Individual data of the* (**A**) *net potassium flux* (J_K) *and* (**B**) *transmural voltage* (V_T) *of the MTAL segments isolated from the kidneys of 4 groups of rats.* Closed circles and bars indicate means and SE.

ranged from 36 to 52 mV with a mean of 43.5 mV, and the deflection of the apical membrane voltage upon luminal potassium change (ΔV_A) ranged from 1 to 18 mV with a mean of 7.9 mV. Seven minority cells were comparable to LBC cells in the hamster MTAL [5, 6], having a low basolateral and a high apical membrane potassium conductance (Fig. 3A). In these cells, ΔV_B ranged from 3 to 18 mV with a mean of 7.1 mV, and ΔV_A ranged from 42 to 48 with a mean of 44.1 mV. The distinction of two cell populations became more apparent when ΔV_A was plotted against ΔV_B (Fig. 3). Thus, the HBC is defined as $\Delta V_A < 20$ mV and $\Delta V_B > 30$ mV, whereas the LBC is defined as $\Delta V_A > 40$ mV and $\Delta V_B < 20$ mV.

Effect of adrenalectomy

All 28 cells from 20 tubules from ADX rats had low basolateral membrane potassium conductance, with ΔV_B ranging from 3 to 18 mV. According to the criteria mentioned above, we must admit that there was no HBC on the basis of the range of ΔV_B. However, we observed that ΔV_A in this group distributed more widely than in the control, ranging from 26 to 56 mV. Twenty-one cells out of 28 cells had ΔV_A less than 40 mV. Therefore, we considered that these 21 cells belong to HBC. Under this circumstance, the HBC is segregated from the LBC by the difference in the magnitude of ΔV_A; ΔV_A ranged in seven cells from 44 to 56 mV with a mean of 48.1 mV, whereas in 21 cells it ranged from 21 to 36 mV with a mean of 30.6 mV. Figure 3B shows two cell populations by plotting ΔV_A against ΔV_B. Representative tracings of the majority cell and minority cell from ADX group are shown in Figure 2B.

As summarized in Table 3, decreases in the basal level of V_B was observed by ADX in both the HBC and LBC. ADX decreased ΔV_B and increased ΔV_A of the HBC without affecting these parameters of the LBC.

Effect of dexamethasone

In the 21 MTALs from the DEX group, 26 cell impalements were conducted. As shown in Figure 3C, there are two cell populations which are comparable to those observed in the control group with respect to ΔV_B and ΔV_A. Six cells had a high apical potassium conductance and a low basolateral potassium conductance, whereas 20 cells had a high basolateral potassium conductance and a low apical potassium conductance. The means for ΔV_A and ΔV_B for each cell type were not different from those in the control group (Table 3). The ratio of HBC/LBC was similar to that in the control. The basal V_B values of both cells were not different from those in the control (Table 3).

Effect of aldosterone

In all 25 impaled cells of 19 tubules isolated from ALDO group, the basolateral membrane potassium conductance was small. The distribution of ΔV_A versus ΔV_B was very similar to that observed in ADX group (Fig. 3D). Although ΔV_A and ΔV_B of the LBC were not different from those of the LBC of the control group, ΔV_A was increased and ΔV_B was decreased in the HBC (Table 3). The basal values of V_B in both cell types were not different from those of the control group (Table 3).

Discussion

Effect of adrenocortical steroids on MTAL and urinary potassium excretion

The kidney is known to be the target of the action of glucocorticoids as well as mineralocorticoids. Although it has been well

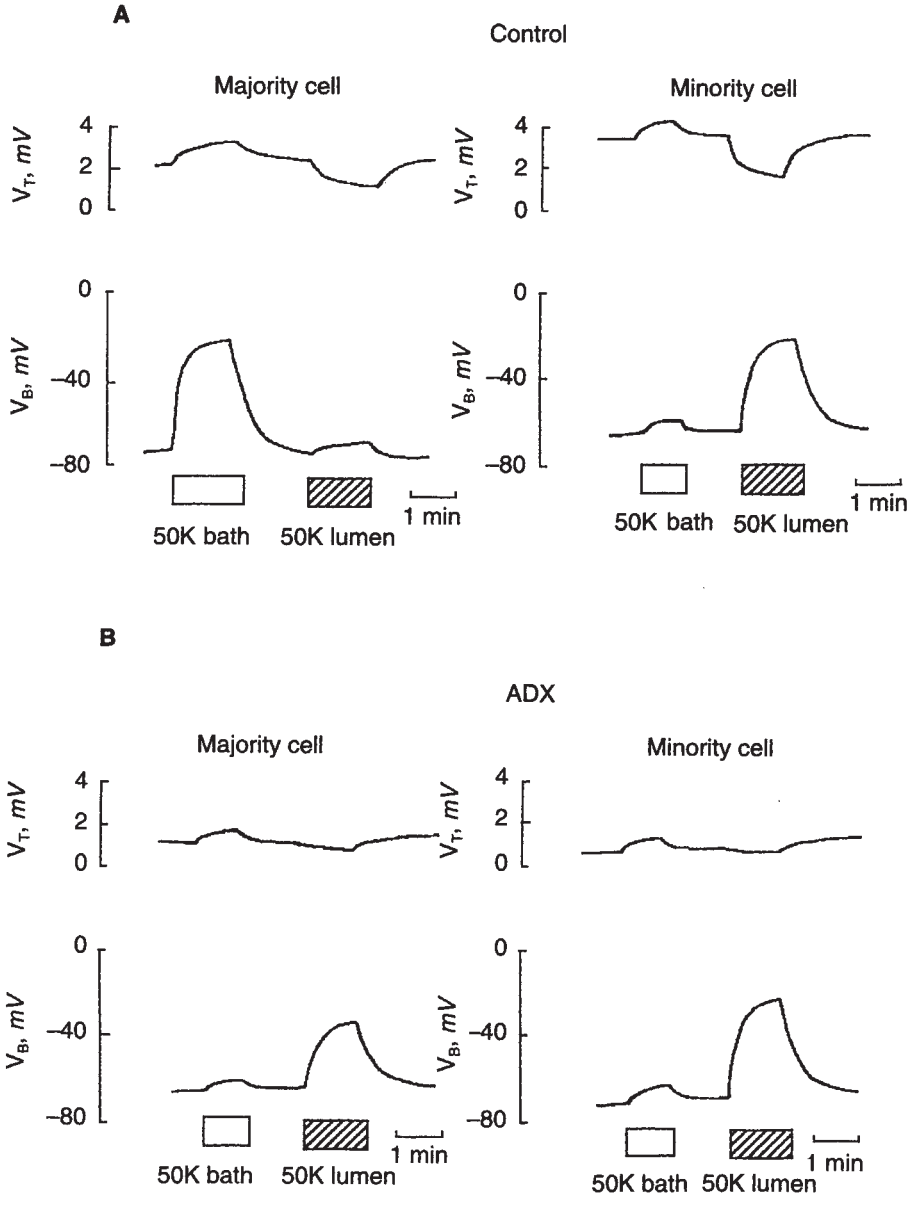

Fig. 2. *Representative tracings of V_T and V_B of a HBC (majority cell) and a LBC (minority cell) of the MTAL isolated from the control* (**A**) *and adrenalectonized rats* (**B**). K^+ concentration either in the lumen or in the bath was abruptly changed from 5 mM to 50 mM.

established that aldosterone increases sodium reabsorption and potassium secretion by mainly acting on the collecting duct [1], the possible action on other nephron segments cannot be ruled out. *In vivo* micropuncture studies in rats [7–10] demonstrated that glucocorticoids act on the Henle's loop. The present study was designed to clarify which of these adrenal steroids play a major role in modulating the potassium transport across the MTAL and which cell type of the MTAL is mainly responsible.

In the clearance study of the whole animal, we confirmed that adrenalectomy increased the fractional excretion of sodium and decreased the fractional excretion of potassium. Although the serum sodium concentration was unchanged, the serum potassium concentration was increased. The altered parameters were partially improved by the treatment with dexamethasone or aldosterone, indicating that the both steroids are responsible for the renal handling of sodium and potassium. As opposed to the clearance data, we found that dexamethasone acts on the MTAL to increase net potassium flux. Adrenalectomy decreased the net potassium flux across the MTAL. The supplementation with dexamethasone, but not with aldosterone, improved the decreased potassium transport.

These findings, however, are at variance with the observation in the *in vivo* micropuncture study by Stanton [10]. He reported that in the rat kidney adrenalectomy decreased potassium flux across the superficial loop of Henle. This decrease was completely recovered by the supplementation with aldosterone, whereas it was partially recovered by the supplementation with dexamethasone. Our results are also at variance with those of *in vitro* microperfusion of the rat MTAL by Work and Jamison [11], in which neither adrenalectomy nor supplementation with aldosterone affected the net potassium flux across the MTAL. At the

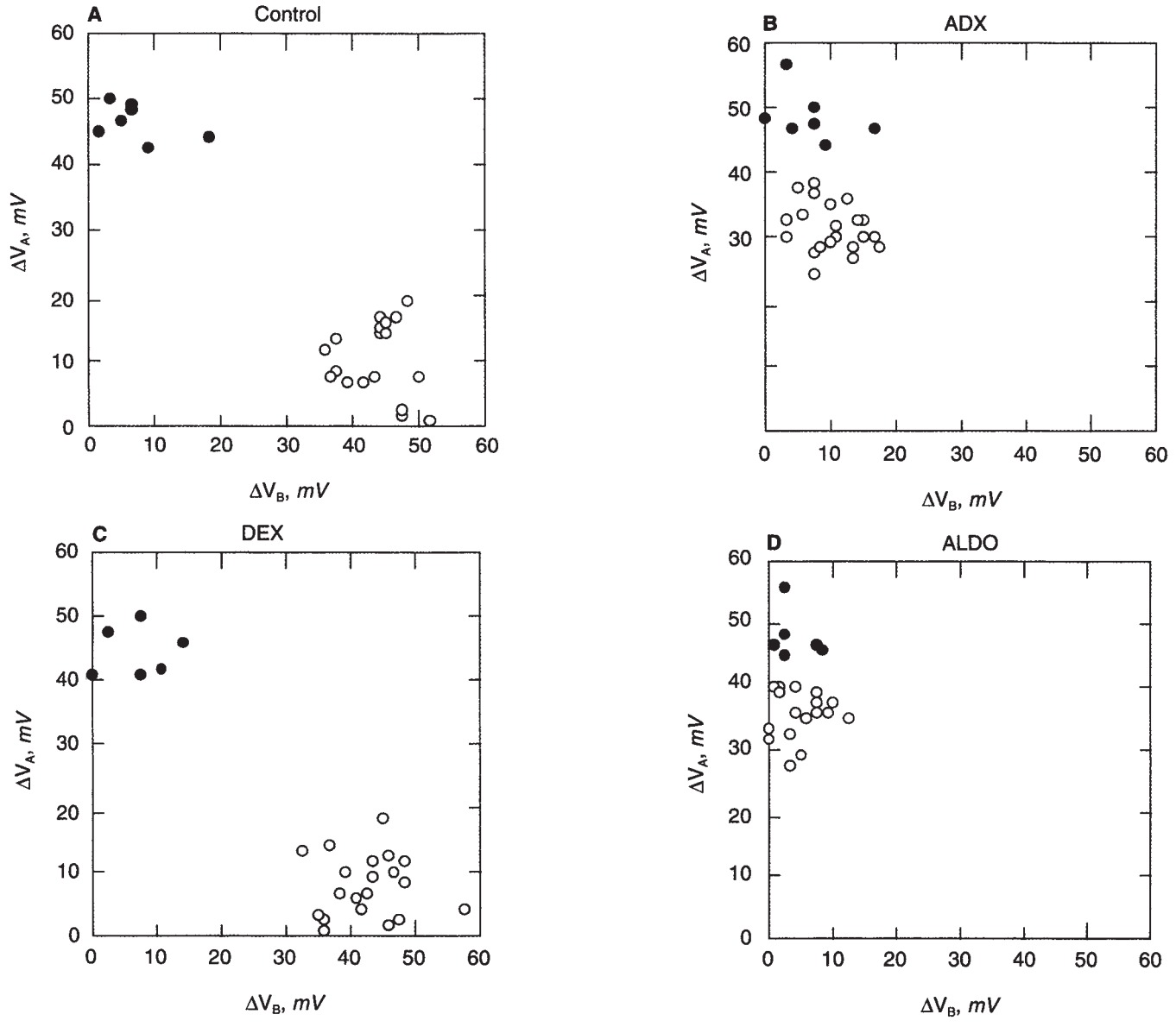

Fig. 3. *Changes in apical and basolateral membrane K^+ conductances in two cell populations of the MTAL isolated from the rats sham operated* (**A**)*, adrenalectomized* (**B**)*, adrenalectomized and supplemented with dexamethasone* (**C**)*, and adrenalectomized and supplemented with aldosterone* (**D**). The deflection of the apical membrane voltage upon abrupt increase in luminal potassium concentration (ΔV_A) was plotted against the deflection of the basolateral membrane voltage upon abrupt increase in bath potassium concentration (ΔV_B). Symbols are: (○) HBC; (●) LBC.

present time, the reason for the discrepancies among these studies including ours are unknown. However, the following points deserve mentioning. Although we used the same dose of aldosterone as reported by Stanton [10], it is possible that the actually available aldosterone was not sufficient to compensate for the deficiency caused by adrenalectomy. In this regard, it should be noted that Stanton [10] continuously applied aldosterone by osmotic minipumps, whereas we bolusly injected aldosterone once a day. Because we used young rats in this study, it is possible that the sensitivity to aldosterone might be low in the young rat. However, because of the lack of direct evidence in support of this notion, a definite conclusion should await further studies.

An apparent discrepancy of the action of glucocorticoids on the potassium transport in the MTAL and that on the potassium balance in the whole animal could be explained by the paradoxical nature of the contribution of potassium transport across the MTAL to the potassium excretion in the final urine as we have previously proposed [6]. Potassium as well as sodium is accumulated in the renal medulla. The potassium recycling taking place in the Henle's loop [2, 3] may play an important role for the accumulation of potassium in the renal medulla. The potassium concentration in the renal medullary interstitium may influence the potassium excretion in the final urine by affecting potassium flux across the medullary collecting duct. Thus, the stimulation of net potassium transport in the MTAL may result in an increase in potassium concentration of the medullary interstitium, which in

Table 3. Summary of electrophysiological studies in two cell types from the MTAL from four groups of rats

	Control	ADX	DEX	ALDO
HBC	(18)	(21)	(20)	(17)
V_T	2.7 ± 0.2	1.5 ± 0.1[a]	3.3 ± 0.1[ab]	1.6 ± 0.2[a]
V_A	−78.0 ± 1.8	−68.0 ± 2.2[a]	−81.9 ± 1.1[b]	−74.4 ± 1.4
V_B	−75.3 ± 1.8	−66.5 ± 2.0[a]	−78.6 ± 1.1[b]	−72.8 ± 1.3
ΔV_A	7.9 ± 1.1	30.6 ± 1.5[a]	7.6 ± 1.1[b]	35.0 ± 0.9[a]
ΔV_B	43.5 ± 1.1	10.0 ± 0.8[a]	42.9 ± 1.3[b]	4.5 ± 0.8[ab]
LBC	(7)	(7)	(6)	(7)
V_T	2.5 ± 0.3	1.3 ± 0.2[a]	3.3 ± 0.3[ab]	1.6 ± 0.2
V_A	−80.4 ± 3.6	−67.9 ± 4.2[a]	−78.5 ± 1.9[b]	−73.0 ± 2.0
V_B	−77.9 ± 2.8	−66.6 ± 3.4[a]	−75.2 ± 1.7	−71.4 ± 1.9
ΔV_A	44.7 ± 1.0	48.1 ± 1.5	44.5 ± 1.6	47.5 ± 1.4
ΔV_B	7.1 ± 2.1	7.3 ± 1.8	6.0 ± 1.9	3.7 ± 0.9[b]

Abbreviations are: HBC, high basolateral conductance cell; LBC, low basolateral conductance cell; ADX, adrenalectomy; DEX, dexamethasone; ALDO, aldosterone, V_T, transmural voltage; V_A, apical membrane voltage; V_B, basolateral membrane voltage; ΔV_A, deflection of apical membrane voltage elicited by an abrupt increase in luminal K^+ concentration from 5 to 50 mM; ΔV_B, deflection of basolateral membrane voltage elicited by an abrupt increase in bath K^+ concentration.

[a] $P < 0.05$ compared to values in the control

[b] $P < 0.05$ compared to values in ADX

turn decreases potassium reabsorption across the medullary collecting duct, leading to an increase in potassium excretion in the final urine.

Sites and mechanisms of action of glucocorticoid in the MTAL

Our observation suggests that glucocorticoids increase the lumen positive V_T of the MTAL. If this change in V_T reflected primarily an increase in the back diffusion of cations through the paracellular pathway, the net potassium flux would have been decreased rather than increased. As will be discussed subsequently, an increase in the apical membrane potassium conductance might account for the increase in the lumen positive V_T. However, the possible contribution of changes in the basolateral chloride conductance has not been tested in this study. At any rate, the increase in lumen positive V_T is also favorable for the potassium absorption through the paracellular pathway [14].

Yoshitomi et al [5] reported that there are two cell populations in the hamster MTAL. In the present study, we confirmed that the similar cell heterogeneity also exists in the rat MTAL. The ratio of HBC/LBC observed in this study was similar to that in the hamster MTAL. More recently, Tsuruoka et al [6] reported that potassium is absorbed in the hamster MTAL which is dominated by the HBC cells. In the present study, we confirmed that the same holds true in the rat MTAL. The J_K obtained for the rat MTAL was comparable to that obtained in the hamster MTAL. Thus, it seems that the HBC or MJC of the rat MTAL is the potassium reabsorbing cell.

The most unique finding of the present study is that glucocorticoids mainly, if not exclusively, act on the majority cell of the MTAL. We have previously defined this cell type as the HBC cell [5, 6, 13]. However, the results of the present study showed that the HBC in the ADX group was not any more the HBC cell as defined by an apparent basolateral membrane potassium conductance. In all cells of the ADX group, ΔV_B on potassium concentration challenge was less than 20 mV. In this sense, there were no HBC cells. However, based on the distribution of ΔV_A, we noted that there were two cell populations, one having a high apical potassium conductance and the other having a relatively low apical potassium conductance. This finding raises the following three possibilities. First, adrenalectomy affects the HBC, decreasing the basolateral potassium conductance and increasing the apical potassium conductance. Second, adrenalectomy caused the conversion of phenotype of the HBC to the LBC. And third, adrenalectomy affects the LBC, causing a decrease in the apical potassium conductance. The last possibility, however, is unlikely, because if this is the case, one cannot explain why the HBC is completely absent. It is difficult to distinguish between the first and second possibilities. However, because of the persistence of two cell populations as determined by ΔV_A, we think that the first possibility is more likely.

In the HBC of the ADX group, the basolateral membrane potassium conductance is decreased and the apical membrane potassium conductance is increased. These findings are consistent with the observation that the net potassium flux was decreased in the MTAL of ADX rats. It is reasonable to assume that under this circumstance the potassium secretion across the apical membrane is increased and that the potassium exit across the basolateral membrane is decreased. DEX reversed the changes in potassium conductances to the control levels, whereas ALDO had no effect. These observations strongly suggest that glucocorticoids play a major role in the regulation of potassium conductances in the HBC of the rat MTAL.

In ADX animals, serum potassium concentration is elevated and the animals are suffering from metabolic acidosis. Let us consider whether these two pathological conditions are responsible for the observed changes. Guggino [4] reported that in the amphibian diluting segment the ratio of HBC to LBC changed when the animals were kept in high potassium environment. In the present study, the profile of potassium conductances were quite different between DEX and ALDO groups, even though serum potassium concentrations were similarly increased. Therefore, serum potassium concentration alone does not account for the changes in potassium conductance of the MJC in our experimental conditions.

It is known that acidosis decreases potassium conductances of the collecting duct [15, 16]. Therefore, it is also possible that acidosis caused by adrenalectomy is responsible for the observed changes in potassium conductance of the HBC of the MTAL. The systemic acidosis by adrenalectomy is mainly caused by the lack of aldosterone. Although we did not measure plasma pH under our experimental condition, both ADX and DEX groups may be suffering from systemic acidosis, whereas acidosis may be improved in ALDO group. Yet, the patterns of potassium conductance profiles are not consistent with the state of acidosis. Stanton [10] measured blood pH in the rats of control, ADX, DEX, and ALDO groups, under the conditions comparable to the present study. He reported that there were no significant differences in blood pH among these groups. Wang et al [17] reported by the patch clamp study that the K^+ with a small conductance present in the apical membrane of the rabbit MTAL was unaffected by pH. Therefore, it is unlikely that metabolic acidosis is mainly responsible for the observed changes in potassium conductances in the HBC.

It has been reported that dexamethasone [18–20] but not aldosterone [19] increased Na, K-ATPase activity in the MTAL. It

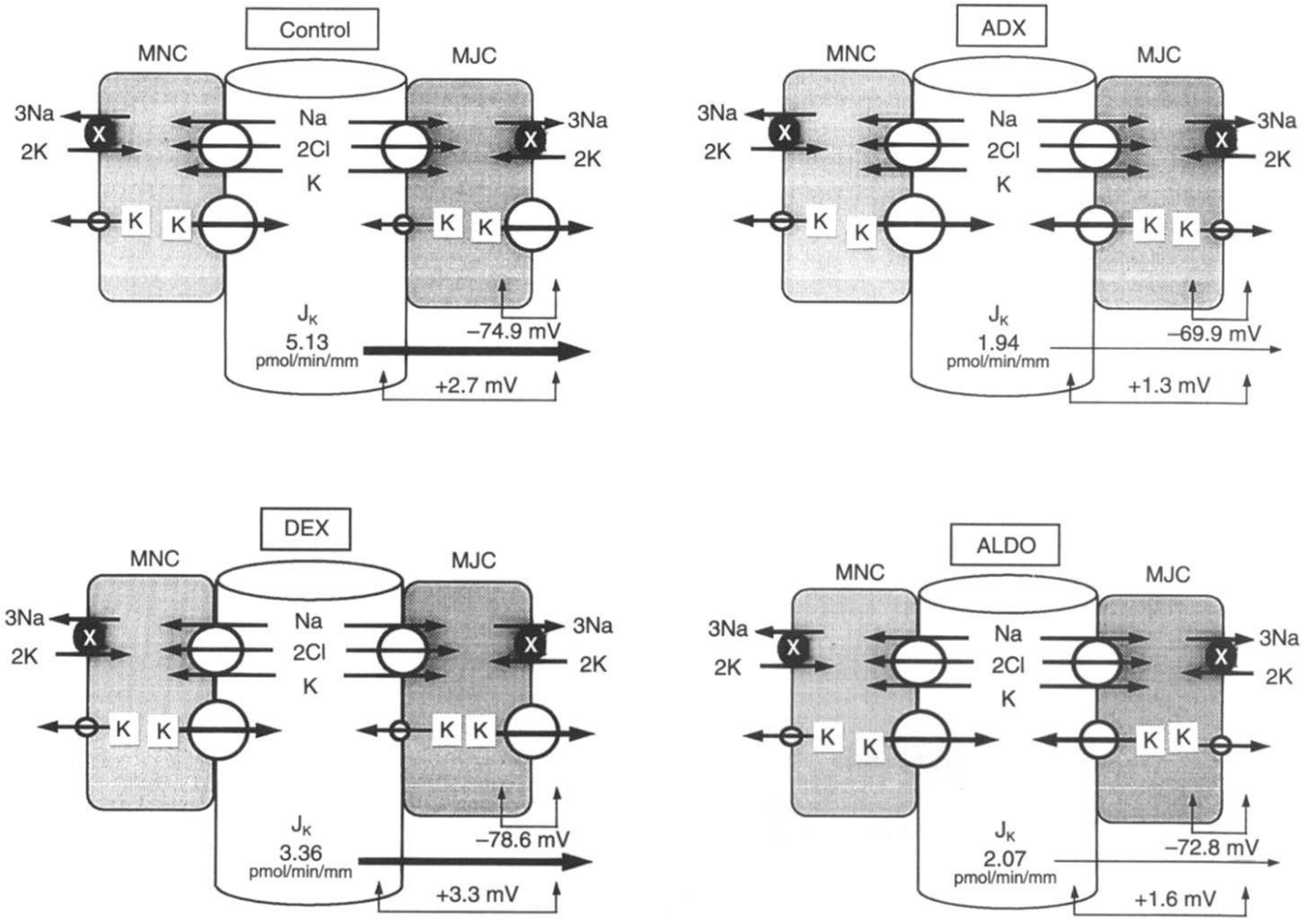

Fig. 4. *Schematic illustration of the summary of the data of this study.* For simplicity, Cl^- channels and KCl cotransporters are not shown. The size of circles roughly represents the magnitude of the K^+ conductances. Abbreviations are: MJC, majority cell (= HBC); MNC, minority cell (= LBC).

is possible that the reduction of the basolateral potassium conductance is secondarily associated with the reduction of Na, K-ATPase. Ouabain is known to inhibit Na, K-pump and decrease V_B [21]. In our experiments V_B was significantly decreased in adrenalectomized animals.

It has been reported that mineralocorticoid receptors and their mRNA are less prominent in the MTAL [22–25]. This is consistent with our findings that glucocorticoids mainly modulate potassium transport in the MTAL. It has also been reported that mineralocorticoids at a high concentration bound to glucocorticoid receptors which are abundant in the MTAL [24]. Therefore, it is possible that mineralocorticoids at high doses may also influence potassium transport in the MTAL. However, it is reasonable that ALDO group did not show any improvements of changes in potassium transport induced by adrenalectomy, because serum aldosterone concentration of this group was within normal level. On the other hand, it has been reported that in the amphibian diluting segment aldosterone, but not dexamethasone, increased the apical potassium conductance via activation of Na/H antiporter and cell alkalinization [26]. In the same preparation, it was reported that aldosterone increased net potassium transport [27]. Whether these findings are due to species difference remains to be established.

Roles of alsdosterone

Although our data clearly indicate that aldosterone may have no direct action on the HBC, it is possible that changes in plasma ion concentrations caused by aldosterone may influence the function of the MTAL. In this regard, it is of interest to note that in the ALDO group the basolateral membrane K^+ conductance in both cell types was lower than that in the ADX group. The improvement of systemic hyperkalemia by aldosterone might account for this phenomenon. Further supportive evidence for the indirect effect of aldosterone is the finding that the recovery of J_K in DEX group was not complete in spite of the complete recovery of K^+ conductances in HBC. It is possible that the basolateral membrane K^+ conductances are influenced by the plasma K^+ concentration.

Summary and conclusion

Essential features of the findings of the present study are illustrated in Figure 4. Glucocorticoids may increase potassium

reabsorption across the MTAL by acting mainly on the HBC, where the potassium conductance in the apical membrane is decreased and that in the basolateral membrane is increased by glucocorticoids. This may cause an increase in potassium concentration in the renal medulla, leading to an inhibition of potassium reabsorption across the medullary collecting duct. Thus, glucocorticoids may have cooperative action on the urinary potassium excretion with mineralocorticoids, which increase potassium secretion by mainly acting on the collecting duct cell.

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